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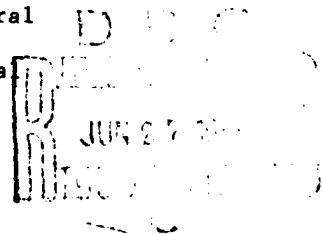
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IMMUNOFLUORESCENT TESTS AND THEIR SIGNIFICANCE
IN CONTROLLING DYSPEPSIA COLI

Orvosi Hetilap
(Medical Weekly)
No. 106, 1965, pages 1171-2

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Dr. Gizella Gago, and
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Infections of dyspepsia coli (in the following: dysp. c.) constitute a serious problem in infants' wards' infants' homes, and in nurseries. The seriousness of the problem derives to a great extent from the fact that bacteriological testing of the stool requires several days, and in the meantime the disease may spread in the ward section where the sample was taken.

Infections arising in this way appear occasionally in scattered instances, and occasionally in mass occurrences, and comprise a serious danger to both healthy infants and infants suffering from other diseases. It is a well-known fact that this infection not only disturbs the development of the infant and aggravates the existing disease, but constitutes a direct danger of life. This danger applies primarily to infants during the first three months, infants with atrophy and dystrophy, and to premature babies.

Unfortunately, the bacteriological test generally used at present yields positive results only after two or three days, and the results are positive only in a relatively small percentage of individuals actually infected.

On the basis of data contained in the literature (1,2) and domestic experience an attempt was made to compare bacteriological culturing with the immunofluorescent method, which is being applied in increasingly extensive fields, in connection with infants under one year of age admitted to the Tolna Megye Hospital (Tolna megyei Kórház) that were suspected

of having dysp. c.

It was planned to eliminate the well-known shortcomings of the bacteriological culture method by reduction of the test time to several hours, and through the high degree of specificity of the immunofluorescent test (in the following: I.F.T.).

Withaker (3) first used the I.F.T. for identification of dysp. c. and screening of bacterial hosts in 1958. Several other researchers subsequently verified the suitability of this test method for direct identification of dysp. c. in feces (4, 5).

Method

Classic cultures of stool material were made according to the procedure described in the book by P. Balint: Klinikai laboratoriumi diagnosztika (Clinical Laboratory Diagnosis) (6). The I.F.T. method, which first was suggested by Coons and Kaplan (7) for the purpose of bacteriological diagnosis, was applied on the basis of our previous experience (8).

The principle of the method is that certain fluorochromes are bound strongly to serum globulins with antibody action, the antibodies marked in this way are brought into contact with homologous bacteria fixed to an objective slide, and fluoresce intensely under ultraviolet light. No bond may be formed with the antigen in the case of heterologous bacteria, the marked antibodies may be washed off, and no fluorescence is observed.

The indirect method was used in our work. The high-titer rabbit antiglobulin was produced in goats, conjugated with fluoresceineisothiocyanate, and dysp. c. polyvalent immunosera produced in rabbits were used as antibodies, which were furnished by the National Public Health Institute (Országos Kózegegysegügyi Intézet).

The test feces was diluted 5- to 10-fold with 0.85 percent NaCl solution, depending on consistency, the pH of the suspension was adjusted to 8.0, and following sedimentation a smear was made from the supernatant. According to our experience with this new smear preparation procedure the bacteria fluoresce intensely in the case of homologous bonds, while other portions of the feces show only dim auto-fluorescence.

Results

Patients for the I. F. T. test were selected from a ward section in which dysp. c. occurs almost constantly. At the time of the test a large epidemic was taking place in the ward which, according to bacteriological tests was caused in most cases by the B₄ strain.

A total of 114 infants with diarrhea was tested at the same time, with the traditional bacteriological and I.F.T. methods. Stool samples were taken from each admitted infant daily during the first three days, and later at five-day intervals; thus 641 samples were processed at approximately the same time.

Table 1.

Comparison of Results of Bacteriological Cultures and the Immunofluorescence Test

BV			IFV			KK		
+	26	22.8%	+	90	78.9%	+	101	88.6%
-	88	77.2%	-	24	21.1%	-	13	11.4%
(1) Össz. 114 100%			Össz. 114 100%			Össz. 114 100%		

BV = bakteriológiai vizsgálat
IFV = immunfluoreszcens vizsgálat
KK = klinikai kép

Key to Table:

BV - Bacteriological Test; IFV - Immunofluorescence Test;
KK - Clinical Picture; (1) Total.

The results of the bacteriological test (in the following, BT) are compared with those of the I.F.T. in Table 1. It was found that the I.F.T. gave positive results in 78.9 percent of the cases, compared to 22.3 percent for the BT. These results were verified by the clinical picture. In the positive cases loose stool, severe diarrhea, stagnation of weight, occasionally loss of weight, loss of appetite, etc. were noted. In no case was the BV positive, and the I.F.T. not. Other enteric diseases were not processed in the investigation.

The positive culture results of tests made in the Szekszárd KOJAL (Közegészségügyi és Járványügyi Allomás; Public Health and Medical Clinic for Contagious Diseases) Laboratory agreed in every case with the positive indications of the I.F.T. Of the samples found to be negative by the culture method 56.1

percent were found to be positive by the I.F.T.

On the basis of a breakdown of the infected infants according to disease groups and comparison of the clinical pictures we came to the conclusion that the infants examined by us who were one year or younger were equally susceptible to dysp. c. According to our experience the clinical course of the disease becomes increasingly mild as the end of the first year of age is approached. Our examinations were not extended to infants over one year of age.

Table 2.

Comparison of the Results of Bacteriological
Cultures and Immunofluorescent Tests According
to Age Groups

	(1) Age	(2) Bv	(3) Bv	(4) Bv
(5)	0-3 mo	+ 12 21.5%	+ 38 77.0%	+ 44 89.7%
(6)	3-6 mo	- 37 75.5%	- 11 22.5%	- 5 10.3%
		+ 8 15.7%	+ 42 82.3%	+ 45 88.3%
(7)	6-12 mo	- 43 81.3%	- 9 17.7%	- 6 11.7%
		+ 6 12.9%	+ 10 17.4%	+ 12 25.7%
(8)	Over-all	- 5 57.1%	- 4 28.6%	- 2 14.3%
		114	114	114

Key to Table:

(1) Age; (2) Bacteriological Test; (3) I.F.T.; (4) Clinical Picture; (5) 0 to 3 Months; (6) 3 to 6 Months; (7) 6 to 12 Months; (8) Total.

Parallel tests were made of the stool of infants who could be considered clinically cured. It was found that of the clinically cured 114 infants with diarrhea, who had shown negative bacteriological results at the time of discharge from the hospital, the I.F.T. gave positive results in 19 cases. These later were found to be symptom-free bacterial dischargers.

Discussion

On the basis of the results obtained it is evident that the I.F.T. gives two- to threefold more positive results than the bacteriological culture method. This finding is supported by the above cited literary data. The greater than average rate of positive results obtained by

culturing and by the I.F.T. may be explained by the fact that clinically typical cases were processed. It is an undeniable advantage of the I.F.T. that a diagnosis may be pronounced within one or two hours of taking the feces sample, which for a few days is not influenced by a previously performed antibiotic treatment. Another advantage of the method is the disappearance from the disease causative agent in question from the stool could be established at five-day intervals with the I.F.T.

The possibility of rapid diagnosis of infants who were found to be cured on the basis of clinical symptoms and the I.F.T. results in the case of hospital superinfections was very convincing. In these cases, however, the I.F.T. revealed the appearance of another type of dysp. c. within a few days, which was verified by the clinical state forming soon thereafter.

With the I.F.T. method it was established that 19 of the treated infants remained symptom-free dischargers who, after the applied antibiotic was eliminated from the organism, again returned to the hospital. Positive results were obtained in 18 cases with the I.F.T., and in 4 cases with the EV, and of these two infants died.

According to our experience, in laboratories that have a fluorescence microscope, and suitable serum and good conjugate are available, with the acquisition of a certain degree of experience the simplicity, rapid execution and high degree of specificity of the I.F.T. may offer significant aid to the clinician in the rapid establishment of a diagnosis and in investigation of the patient's environment and the bacterial host. Of course, the determination of antibiotic sensitivity may be achieved only through culturing.

Summary: A total of 114 infants under one year of age showing diarrhea was examined for dyspepsia coli infection, using both the traditional bacteriological method and immunofluorescence. A total of 641 samples was processed. The I.F.T. gave 78.8 percent positive results, compared to only 22.3 percent with the bacteriological test. Comparison of the two test methods according to age groups showed that up to the age of one year the infants examined were approximately equally susceptible to dysp. c. infection. Through the immunofluorescent test we were able to follow the formation of bacteria dischargers. Upon comparison of the two test methods it was found that the immunofluorescent test reduced the necessary time to two or three hours and gave threefold more frequent positive cases, thereby convincingly supporting the work of the doctor.

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